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The Swedish Patent Office PCT International Application

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CLAIMS

- 1. A method for the determination of a tetracycline in a sample <u>characterized</u> in that
- the sample is brought into contact with prokaryotic cells encompassing a DNA vector including a nucleotide sequence encoding a light producing enzyme under transcriptional control of a tetracycline repressor and a tetracycline promoter,
- detecting the luminescense emitted from the intact cells, and
- comparing the emitted luminescence to the luminescence emitted from cells in a control containing no tetracycline
- wherein a detectable luminescence higher than a luminescence of the control indicates the presence of tetracycline in the sample.
- 2. The method according to claim 1 <u>characterized</u> in that the cells are *Escherichia coli*.

The method according to claim 1 state characterized in that the DNA vector is a plasmid containing the luxCDABE genes (SEQ ID NO: 3), tetracycline repressor (TetR) (SEQ ID NO: 11) and tetracycline promotor (TetA) (SEQ ID NO: 9) from Tn10.

- 4. The method according to claim 3 characterized in that the DNA vector is the plasmid pTetLux1 (SEQ ID NO: 3).
- 5. The method according to claim 1002 characterized in that
- the DNA vector is a plasmid containing the insect luciferase gene (SEQ ID NO: 1), tetracycline repressor (TetR) (SEQ ID NO: 11) and tetracycline promotor (TetA) (SEQ ID NO: 9) from Tn10, and that

- D-luciferin is added to the mixture of the sample and the cells in order to initiate the lumines dence of the cells.
- The method according to claim 5 characterized in that the DNA vector is the plasmid pTetLuc1 (SEQ ID NO: 1).
- ims 1 = 6 characterized in that the The method according to any of sensitivity of the analysis with respect to the tetracycline is controlled by
- increasing or decreasing the concentration of divalent metal ions, e.g. magnesium ions, or
- adjusting the pH, or
- combined adjusting δf the divalent metal ion concentration and the pH.
- The method according to any of the ms-1=6 characterized in that the sensitivity of the analysis with respect to the tetracycline derivative is increased by the use of cells which are especially antibiotic sensitive mutant strains.
- e-claims 1 8 characterized in that the The method according to any of luminescence is measured using an X-ray or polaroid film, a CCD-camera, a liquid scintillation counter or a luminometer.
- 10. The method according to any of the claims 1 = 9 characterized in that the sample to be analyzed is milk, fish, meat, infant formula, eggs, honey, vegetables, serum, plasma, whole blood or the like.
- 11. A recombinant prokaryotic cell characterized in that it encompasses a DNA vector including a nucleotide sequence encoding a light producing enzyme,

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tetracycline repressor and tetracycline promoter, and that the DNA vector is a plasmid containing the luxCDABE genes (SEQ ID NO: 3), tetracycline repressor (TetR) (SEQ ID NO: 11) and tetracycline promotor (TetA) (SEQ ID NO: 9) from *Tn*10.

12. The cell according to claim 11 characterized in that it is Escherichia coli.

13. The cell according to claim 1 k characterized in that it is in dried form, e.g. in lyophinzed form.

14. A plasmid <u>characterized</u> in that it comprises the luxCDABE genes (SEQ ID NO: 3), tetracycline repressor (TetR) (SEQ ID NO: 11) and tetracycline promotor (TetA) (SEQ ID NO: 9) from *Tn*10.

15. A plasmid according to claim 14 <u>characterized</u> in that it is pTetLux1 (SEQ ID NO: 3).